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Microsatellite based genetic relationships in the genus *Camellia* – potential for improving cultivars.

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Abstract

There is a lack of published microsatellite data which characterizes *Camellia* spp. To address this, an initial study of Sequence Tagged Microsatellite Site (STMS) variation was undertaken with 132 accessions of *Camellia* spp. which included 25 accessions representing 24 different species, and 63 cultivars of *C. japonica*, 33 of *C. sasanqua*, 7 of *C.* x *vernalis*, 3 of *C.* x *hiemalis*, and 2 of *C. hybrida*. The four primer sets used (MSCJAF37, MSCJAH46, MSCJAF25 and MSCJAH38) successfully amplified polymorphic alleles in all the species analyzed, showing cross-transferability. Overall, 96 alleles were scored. MSCJAH38 primer pairs produced the highest number of bands (30), while MSCJAH46 yielded the least number (15). The genetic distance between pairs of accessions was estimated on the basis of the Nei coefficient and a Principal Coordinate Analysis (PCoA) was computed. The plot revealed a main differentiation between the *C. japonica* cultivars and the winter camellias. The distribution of the genetic variation, attributed by AMOVA, particularly highlighted genetic overlapping among *C. sasanqua* cultivars and the hybrids belonging to *C.* x *vernalis*, *C.* x *hiemalis* and *C. hybrida*. In conclusion, this study demonstrated that STMSs offer a suitable method for detection of genetic variability and molecular study of camellia genotypes.

Additional key words: C. japonica, *C. sasanqua*, STMS, fingerprint, AMOVA, allele frequencies, germplasm characterization.

Introduction

The genus *Camellia* L. belongs to the section *Gardonieae* of the family *Theaceae* Mirbel (Sealy 1958) and comprises more than 325 species (Mondal 2002). Taxonomical problems are mainly due to natural inter specific hybridization occurring in the genus. All the naturally occurring species and hybrids are distributed in the south-eastern regions of Asia, from Himalaya to Japan and from southern China (Guangxi and Yunnan) to Java and Sumatra. The chromosome set is basically diploid (2n=2x=30; Kondo 1977), such as in *C. sinensis* (L.) O. Kuntze (Morinaga et al. 1929) and *C. japonica* L. (Morinaga and Fukushima 1931). However, polyploidy could be found in several species: *C. sinensis* (2n=3x=45; Karasawa 1932), *C. japonica* (2n=2x=30, 2n=3x=45; Janaki-Ammal 1952), *C. fraterna* Hance (2n=3x=45; Longley 1956), *C. sinensis* var. *sinensis* f. *macrophylla* (Sieb.) Kitamura (2n=3x=45; Janaki-Ammal 1953 and 1956), *C. granthamiana* Sealy (2n=4x=60; Fukushima et al. 1966), *C. reticulata* Lindley (2n=6x=90; Janaki-Ammal 1952), *C. sasanqua* Thunberg (2n=5x=75, 2n=6x=90, 2n=7x=105, 2n=8x=120; Ito 1957; Janaki-Ammal 1953 and 1956), *C. oleifera* Abel (2n=4x=60, 2n=6x=90; Patterson et al. 1950, Ackerman 1971), *C.* x *hiemalis* Nakai (2n=6x=90; Ito et al. 1955) and *C.* x *vernalis* Makino (2n=3x=45; Ito et al. 1955).

Several species have economic importance. The first report concerning the cultivation of *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* for the production of tea dates back to 500 B.C (Eden 1958). For ornamental value, *C. japonica* is the main widespread commercial species, with thousands of different cultivars. From the beginning of the 20th century, *C. sasanqua* also started to be promoted in Europe for its winter blooming (Scariot et al. 2009). While it has been long appreciated in Japan and China, only in the 18th century this species was recorded by Kaempfer in his *Amoenitatum Exoticarum* (1712).

C. japonica and *C. sasanqua* are both native species of Hirado Island, Japan (Scariot et al. 2009). This area seems to be the origin place of *C.* x *vernalis*, as many variants of *C. japonica* and *C. sasanqua* are present (Uemoto et al. 1980). As reported by an old Japanese book published in the Edo period (1603-1868) *C.* x *vernalis* 'Gaisen' was generated about four hundred years ago from the cross between *C. sasanqua* x *C. japonica*. The hybrid origin was confirmed by cpDNA analysis, which indicated a gene flow of the cytoplasm of *C. sasanqua* into *C.* x *vernalis* (Tanaka et al. 2005). As well as *C. sasanqua* and *C.* x *vernalis*, *C.* x *hiemalis* and

C. hybrida are two other interesting ornamental fall-blooming species traded in the mid- XXth century in Europe and North America. Originated in Japan, *C.* x *hiemalis* is a hybrid between *C. sasanqua* x *C. japonica* (Kondo 1976; Parks et al. 1981). This hybrid form shows numerous similar characteristics (blooming period and flower shape) with *C. sasanqua* (Scariot et al 2009). Moreover, this species was used for interspecific crosses together with selected *C. sasanqua*, and *C.* x *vernalis* cultivars. Breeding projects mainly aimed to combine the desirable ornamental qualities of these species with plant characteristics and hardness of *C. oleifera* (Ackerman and Egolf 1992).

The knowledge of the extent of diversity and relationships within and among species and their wild relatives is essential for the efficient use of plant genetic resources. Erosion can have an alarming effect on the stability of those species in which genetic improvement resulted in an extremely high number of cultivars (Reed and Frankham 2003). Traditionally morphological data (i.e. leaf architecture, growth habitus and floral biology), used as keys for *taxa* characterization, have long played an important role for several classifications. Corneo et al. (2003) investigated historical documents to compare the reported descriptions. Molecular methods were employed with the aim to provide support for identification of discrete taxonomic groups. Interesting discrimination perspectives have been shown by biochemical analysis with isoenzymes (Sànchez-Escribano et al. 1998), but they are limited by the relatively low levels of polymorphism. Molecular markers overcome this problem and have been used to investigate genetic diversity in cultivated plants. In *Camellia*, random amplified polymorphic DNA (RAPD, Chen and Yamaguchi 2002, Young-Goo et al. 2002, Luo et al. 2002, Jorge et al. 2003, Shao et al. 2003), amplified fragment length DNA polymorphism (AFLP, Balasaravanan et al. 2003) markers, inter-simple sequence repeats (ISSRs, Mondal 2002, Freeman et al. 2004), expressed sequence tags (ESTs, Zhao et al. 2007), cleaved amplified polymorphic sequences (CAPSs, Kaundun and Matsumoto 2003), and chloroplast DNA sequences (Prince and Parks 2001, Katoh et al. 2003, Tateishi et al. 2007) were mainly developed to analyse *C. sinensis.* In recent years, Ueno et al. (1999, 2000 and 2002) developed sequence tagged microsatellite site (STMS) markers with the purpose to investigate the genetic structure of wild populations of *C. japonica*.

Among the several classes of DNA-based markers, the STMS are highly polymorphic, multi-allelic, frequently co-dominant, highly reproducible and widely distributed in the genome (Powell et al. 1996). STMS markers are increasingly being used in different crop species for genome mapping and marker-assisted selection (MAS) as well as for germplasm analysis, varietal identification and linkage analysis (Marinoni et al., 2003, Varshney et al., 2005, Krishnan et al., 2005). In the present work, microsatellite allele frequency distribution was employed to describe genetic similarity among 63 *C. japonica* cultivars and 45 cultivars of winter camellias (*C. sasanqua*, *C.* x *vernalis*, *C.* x *hiemalis* and *C. hybrida*) and to assess the genetic relationships among the camellia cultivars and 24 wild species.

Materials and methods

Plant material and DNA extraction

A total of 132 accessions of *Camellia* were selected; these included accessions for 24 different species, in addition to 63 *C. japonica* cultivars, 33 *C. sasanqua* cultivars, 7 *C.* x *vernalis* cultivars, 3 *C.* x *hiemalis* cultivars and 2 *C. hybrida* cultivars (Corneo and Remotti 2003; Accati et al. 2006; Scariot et al. 2007a; Scariot et al. 2009; Table 1). Young leaves were harvested in spring, immediately deep-frozen (-80°C) and reduced to fine powder in liquid nitrogen using mortar and pestle. Total genomic DNA was extracted from approximately 0.2 g of plant material according to Thomas et al. (1993), with some modifications (TRIS-EDTA-NaCl buffer containing 0.25 M NaCl, 0.2 M Tris-HCl pH 7.6, 2.5 % PVP 40.000, 0.05 M Na_2EDTA pH 8.0, 3% Sarcosyl, 20% ethanol and 1% E-mercaptoethanol). The DNA was then dissolved in Tris-EDTA buffer and quantified by spectrophotometer.

STMS amplification and detection of polymorphisms

Four STMS primer sets developed by Ueno et al. (1999) in *C. japonica*, with forward primers labelled with a specific fluorochrome (6-FAM or HEX), were used: MSCJAF37, MSCJAH46, MSCJAF25, and MSCJAH38. The 20 µl reaction volume contained 50 ng template DNA, 2 µl 10X PCR reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM of each primer, and 0.5 U

AmpliTaq Gold® DNA polymerase (Applied Biosystems). Amplifications were performed using the following temperature cycles: initial step of 9 min at 95°C, followed by 28 cycles of 30 s at 95°C, 45 s at 50°C and 1 min and 30 s at 72°C, with a final elongation step of 45 min at 72°C. Samples were then analysed on an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, Calif., USA). Data were processed by the GeneMapper Software 4.0 (Applied Biosystems) and alleles defined by their size (in bp), compared with standard (GeneScan-500 LIZ, Applied Biosystems).

Data analysis

Due to the polyploid nature of samples the STMS bands were scored as discrete variables, using 1 or 0 to indicate the presence or the absence of each fragment (Esselink et al. 2003) and the data were analysed using the SPSS 15.0 package. Allelic frequencies were calculated and summed for ranking the accessions according to their use of alleles (Klein et al. 2008). The genetic distance between pairs of accessions was estimated on the basis of the Nei coefficient (Nei 1978) and a Principal Coordinate Analysis (PCoA) was computed using GeneAlEx 6.3 (Peakall and Smouse 2006). Genetic distances using the *formula* proposed by Nei and Li (1979) were computed on all the 24 different studied species and *C.* x *vernalis* 'Gaisen'. Cluster analysis, applying Neighbor-joining, using arithmetic means (Sneath and Sokal 1973) was applied with the TREECON software (Van de Peer and De Wachter 1994). By applying the same software, the statistical stability of the branches in the tree were estimated by bootstrap analysis with 1000 replicates. Total genetic diversity in variance components was calculated among and within populations, through the Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) at one level with 1000 permutations using AMOVA 1.5 package software.

Results

Relatedness among cultivars and species

Table 1 summarizes all the PCR amplicon sizes of the STMSs in the genepool that could be used for genetic identification of the camellia cultivars. Overall, MSCJAH38 produced the highest number of bands (30),

while MSCJAH46 yielded the least number (15). In *C. japonica* cultivars, the range was from 6 (MSCJAH46) to 17 (MSCJAF37); in *C. sasanqua* cultivars the range was from 7 (MSCJAF25) to 18 (MSCJAF37); in *C.* x *vernalis* cultivars it was from 6 (MSCJAH46) to 14 (MSCJAH38); in *C.* x *hiemalis* cultivars it was from 5 (MSCJAF25) to 8 (MSCJAF37) and in *C. hybrida* cultivars the range was from 0 (MSCJAF25) to 7 (MSCJAF37; MSCJAH38). Not all the STMS were amplified in all the accessions, in particular MSCJAF37 was detected in 97 %, MSCJAH46 in 94 %, MSCJAH38 in 91 % and MSCJAF25 in 87 % of the samples. Many cultivar-specific bands were obtained. The mean observed heterozygosity was 0.119 at each 4 loci indicating low diversity of *Camellia* germplasm. No null alleles were observed (r= -0.05). Therefore, in Table 1 the presence of one single-sized allele indicates homozygosis for such STMS. In order to verify the apportionment of each camellia cultivar to its own horticultural group, the separate frequencies of all alleles for each cultivar were summed (Tab. 1). The highest allele-frequency summation indicates which cultivar belongs most closely to the general population and *vice versa* (Klein et al. 2008). In general, *C. japonica* cultivars showed high values (0.32-0.56). Only for seven cultivars (‘Humilis’, ‘6017’, ‘Amelia Brozzoni’, ‘12B’. ‘Gloria del Verbano’, Mathohiana Rosea’ and ‘Otome-tsubaki’) the sum of allele frequency was lower than 0.32. Similarly, in winter camellias only ‘Totenko’, Hiryu’, and ‘Hina yuki’ showed low values (≤ 0.29). Table 2 summarizes the frequencies of all the loci sizes of the studied cultivars, with the highest frequency for each microsatellite shown.

In order to provide information on the differentiating capacity of each marker, the number of unique banding patterns per assay unit was calculated (Table 3). In all *Camellia* studied genotypes, in *C. japonica*, and in *C. sasanqua* cultivars, MSCJAF37 with 55 (0.41), 26 (0.41) and 16 (0.48) unique banding patterns respectively, outperformed MSCJAH46, MSCJAF25 and MSCJAH38 in discriminating plants. The markers MSCJAF37, MSCJAH46 and MSCJAH38, differentiated all *C. hybrida* cultivars each separately. The *C.* x *vernalis* cultivars were better discriminated (0.71) by means of the primer set MSCJAF25, while *C.* x *hiemalis* cultivars by means of MSCJAH46 with 3 (1.00) unique banding patterns.

Based on the allele matrix reported in Table 1, a PCoA that illustrates the relatedness between all the accessions was constructed (Fig. 1). The Coord. 1 accounted for 50.10 % of variance and revealed a first order

of differentiation between *C. japonica* (A) and winter camellia (*C. sasanqua, C.* x *vernalis*, *C.* x *hiemalis* and *C. hybrida*) (Group B) cultivars. All species were grouped in two intermediate groups (C and D). In particular, *C. japonica, C. japonica* var. *rusticana, C. japonica* var. *intermedia, C. japonica* var. *hozanensis, C. tsaii*, and *C. cuspidata* were slightly intermixed with the group A. Moreover, a group (D) including *C. sasanqua*, *C. reticulata*, *C. caudata, C. granthamiana, C.* x *vernalis* 'Gaisen' was located close to group B.

With the aim to go deeply in a taxonomic comparison of the studied species, a dendrogram based on STMSs data was constructed (Fig. 2). The 25 accessions were divided by medium-low bootstrap values expressed in percentage. The highest value was found between *C. caudata* and *C. sasanqua* (52%). No clear groupings were obtained. Fig. 2 shows the ploidy level deduced from the microsatellite patterns of each species studied. The levels obtained for *C. japonica* var. *simplex*, *C. japonica* var. *hozanensis*, *C. japonica* var. *intermedia*, *C. sinensis* var. *sinensis*, *C. granthamiana, C.* x *vernalis* 'Gaisen', *C. japonica*, *C. fraterna*, *C. japonica* var. *rusticana*, and *C. sinensis* var. *assamica* confirmed literature data (Morinaga et al. 1929; Morinaga and Fukushima 1931; Karasawa 1932; Patterson et al. 1950; Janaki-Ammal 1952, 1953 and 1956; Ito et al. 1955; Longley 1956; Fukushima et al. 1966; Ackerman 1971; Kondo 1977).

To attribute the distribution of the genetic variation, an AMOVA (Table 4) was performed based on the overall data set (a.) and only the cultivars (b.). The results obtained (Tab. 3 a) showed significant differences (p= 0.001, determined from a 1000 replication bootstrap) within populations (66.47 %) and among populations (33.53 %). Moreover, the matrix generated by PhiST between pairs of population (data not shown), revealed the lowest genetic distance between *C. sasanqua* and *C. hybrida* cultivars (0.0765) and the highest distance between *C. japonica* and *C.* x *hiemalis* cultivars (0.5502), demonstrating particular genetic overlapping among *C. sasanqua* cultivars and the *C.* x *vernalis*, *C.* x *hiemalis*, and *C. hybrida* hybrids. Looking at the cultivar data set, 59.91 % of the total variation was attributed to differences within populations and 40.09 % among populations. The pronounced genetic divergence among populations was also highlighted by the value of molecular fixation (Φ_{ST}), which was high (0.401).

Discussion

Marker information

The collection and characterization of genetic resources are of primary importance for the prevention of their loss and for future exploitation. In plant breeding programs, information regarding patterns of genetic variation and relationships within germplasm is crucial for effective decision making. Several powerful PCR-based techniques for DNA fingerprinting are currently available. The choice of the most appropriate technique for any specific study depends principally on the purpose of the research and the genetic structure of the species. Among all the molecular markers, microsatellites are postulated to be useful for marking gene-rich regions (Kojima et al. 1998, Rubeena et al. 2003). Also, STMS markers map to the same locations in both intra- and inter-specific nature, demonstrating that they lie in conserved, evolutionarily stable regions of the genome and may be confidently used for analysis of related germplasms (Vosman et al. 2001, Bredemeijer et al. 2002). Besides, some of the STMS primers developed in one species may be effective in detecting polymorphism in other related species (Scariot et al. 2006). The characterization of microsatellites in *Camellia* (Ueno et al. 1999) provided a co-dominant, highly reproducible and genetically informative marker system (Mondal 2002).

The present work is the first attempt to use molecular markers to characterize such a large cultivarietal germplasm of this genus. The use of the four primer sets resulted in a total of 96 microsatellite alleles for 132 camellia accessions. The fragment sizes were slightly larger than those reported by Ueno et al. (1999). Moreover, most of the STMS markers differed from each other by 1-bp or multiple repeat units.Sometimes this relation was not obvious for all loci, suggesting that other types of sequence variation may also be involved in allele diversity. As detected by Edwards et al. (2008), 1-bp differences could be due to indels in the flanking regions or the repeat region of the microsatellite. The co-dominant nature of STMS markers allows determination of the actual genotype of an individual and estimation of allelic relationships among genotypes. However, co-dominant scoring of the markers in heterozygote samples is complicated in polyploidy individuals (Creste et al. 2003, Esselink et al. 2003). In this study, differences were detected in the amount of amplification products for different alleles of a particular genotype. However, it seemed very difficult to use these differences in a reliable way to estimate whether a particular allele was present in one or more copies, and thus to deduce the effective genotype of an individual. Therefore, the scoring was done in a dominant way and consequently

the band pattern observed did not allow the definition of the allelic genotype. For this reason, the DNA profile of each accession was defined as "allelic phenotype", according to Becher et al. (2000). The four markers for MSCJAF37, MSCJAH46, MSCJAF25 and MSCJAH38 STMS shown sufficient variation for positive distinction between the identities of most camellia cultivars and species. For negative distinction, additional markers may be required to distinguish subgroups of cultivars. Indeed, in this study several cultivars grown in the same conditions, differed phenotypically (petaline shape and variegation colours; Corneo et al. 2003; Scariot et al. 2007a), but showed the same STMS profile. In fact, the cultivars 'Tricolor' and 'Anemonaeflora', 'Vergine di Collebeato' and 'Donckelaeri', 'Amalia Servi' and 'Parvula', 'Kallista' and 'Roma Risorta', and 'Oki-no-nami' and 'Variegata' were typed by the same microsatellite pattern. According to these results, the expression of the shape and colour determinants could be regarded as independent from the STMSs investigated. However, as reported by Klein et al. (2008) the allele sizes derived by the genotyping technology do not guarantee that identical nucleotide sequences must represent identical allele sizes. Amplifying alleles were not successfully scored in all the cultivars, but all STMS primers amplified polymorphic alleles in 19 different camellia species out of 24. This may suggests that these microsatellite loci are conserved within the genus *Camellia* and also means that genomes of the species are homologous (Esselink et al. 2003).

Genetic diversity

According to Ellegren (2004) and Nauta and Weissing (1996), genetic distance measures applied to microsatellite data can yield useful estimators for phylogenetic relationships in closely related populations, as well as in species accessions or cultivars (Hashimoto et al. 2004, Mondal 2002). Results generated by STMSs data are generally in good agreement with the taxonomic classification and pedigree information (Savige 1993, Mondal et al. 2002, Scariot et al. 2006, Scariot et al. 2007b, Tateishi et al. 2007, Ohsako et al. 2008, Caser and Scariot 2009).

In this study, based on PCoA, the results gave a representation of the relationships among cultivars and species, grouping the genotypes into four clusters. Cultivars were divided according to their horticultural classification. Two main clusters were composed by the "japonica" (A) and the winter camellias (B). In the

other two clusters the species and the cultivars with low sum of allele frequencies (Tab. 1) were grouped. In accordance with cpDNA analysis of the *atp*H-*atp*I and *trn*L-*trn*F regions performed by Tateishi et al. (2007), *C. japonica* var. *simplex* and the *C. japonica* var. *hozanensis* clustered separately from *C. japonica*, confirming different glacial evolutionary routes, as shown in the dendrogram in Fig. 2. Moreover, the supposed ploidy levels for ten analysed species (*C. japonica* var. *simplex*, *C. japonica* var. *hozanensis*, *C. japonica* var. *intermedia*, *C. sinensis* var. *sinensis*, *C. granthamiana*, *C.* x *vernalis* 'Gaisen', *C. japonica*, *C. fraterna*, *C. japonica* var. *rusticana* and *C. sinensis* var. *assamica*) obtained by STMS data were in accordance with the literature. All this implies that microsatellites can be useful for preliminary ploidy inference and for establishing relationships between related species and cultivars, taking into account that their level of variability may improve their effectiveness (Alvarez et al. 2001).

The markers developed in *C. japonica* provided interesting results for varietal identification generating subgroups in the *continuum* of winter camellias. In particular, in agreement with the description of Ackerman and Egolf (1992) within the "sasanqua" group were included the *C. hybrida* 'Winter' Star' (*C. oleifera* x *C.* x *hiemalis* 'Showa no Sakae'), the *C.* x *hiemalis* cultivars and, within *C.* x *vernalis*, the so called "Egao" group ('Ginryu', 'Star above star' and 'Vernalis egao'). As mentioned by Tanaka et al. (1987) the "Egaos" originated from processes of back-crossing between *C. japonica* and natural hybrids of *C. japonica* and *C. sasanqua*. Indeed, both *C. japonica* and *C. sasanqua* can be found in the same area on Hirado Island that is probably also the centre of origin of the primary hybrid *C.* x *vernalis* 'Gaisen' which arose around 400 years ago (Uemoto et al. 1980, Tanaka et al. 2005). Moreover, as displayed by the dendrogram and explained by Tanaka (1988) and Sealy (1958) *C.* x *hiemalis* seems to be correlated with *C. sasanqua*. In fact, intermediate cultivars are known which range in morphology, such as blooming season and ploidy level, from typical *C.* x *hiemalis* to typical *C. sasanqua* (Parks et al. 1981).

Analysis of Molecular Variance (AMOVA) was carried out at one level to assess in which percentage the variance could be attributed to genetic differences among and within cultivar groups and species. Most of the genetic diversity was attributable to differences within populations (66.47 % and 59.91 %), reflecting the

extensive genetic variation mainly caused by the origin (breeding company) of the cultivars, which was observed in *C. sinensis* cultivars by Ohsako et al. (2008).

In conclusion, this study demonstrated that STMSs offer a suitable method for detection of genetic variability and molecular study of camellia genotypes. Thanks to their high discrimination capacity, STMSs appeared to be an appropriate tool for establishing relationships between species and cultivars. Moreover, the repeatability of band profiles, the speed of analysis and the high level of polymorphism revealed suggest that this approach has considerable potential for the rapid identification of cultivars (Abe et al. 2003, He et al. 2003, Nybom 2004).

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Figure 1. Principal Coordinate Analysis (PCA) based on the molecular data of 132 genotypes studied.

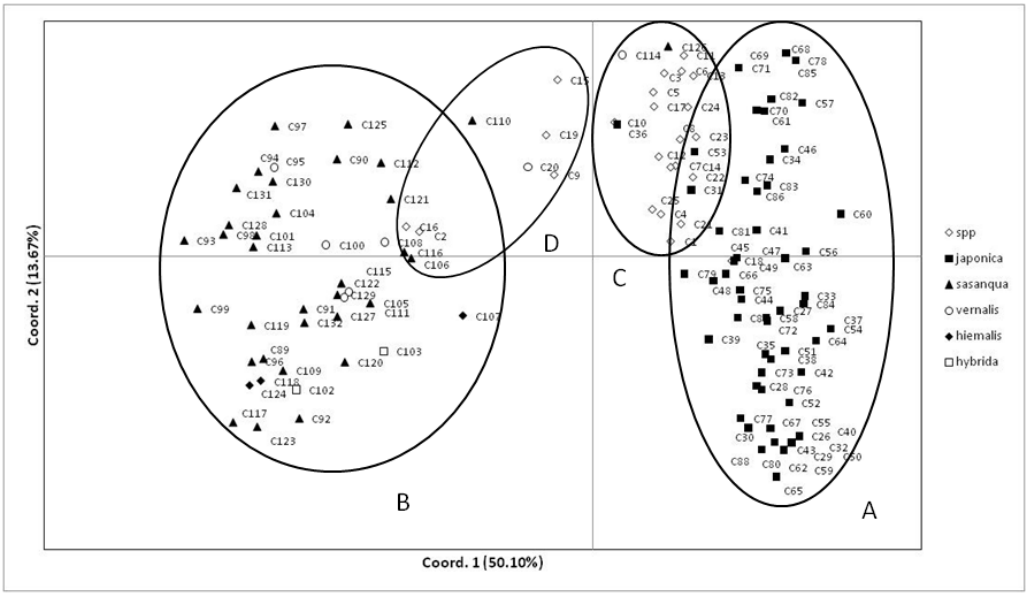

Figure 2. Dendrogram of 25 *Camellia* studied species with the supposed ploidy levels, as obtained from the microsatellite patterns.

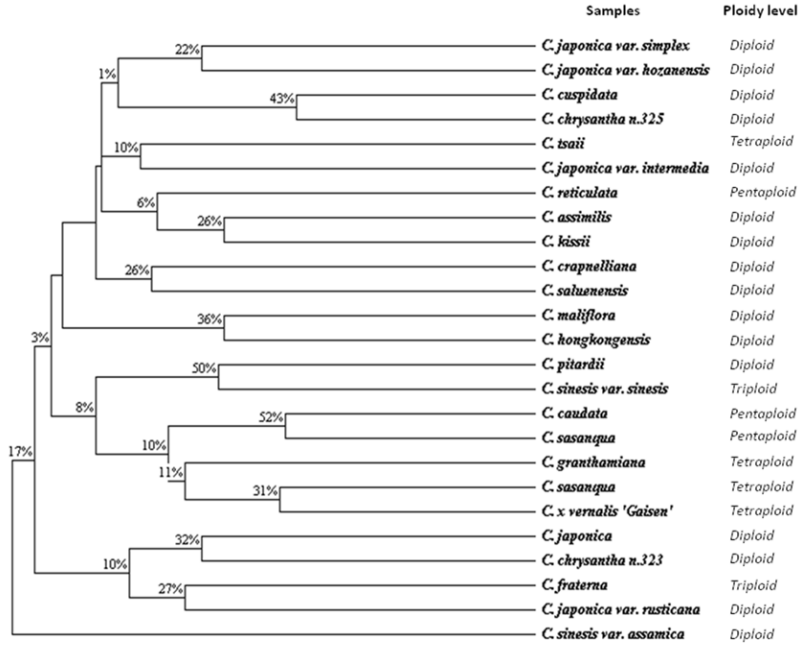

Table 1. Identification code, accession name, origin, allele sizes and sum of allele frequencies of the 132 camellias analysed using microsatellites.

ID	Accession name	Collection site	MSCJAF37	MSCJAH46	MSCJAF25	MSCJAH38	Sum of allele frequencies
C1	*C. fraterna*	Villa Anelli (Vb), Italy	348, 354, 358	446, 448, 458	200*, 217, 225	354, 368	
C2	*C. caudata*	Villa Anelli (Vb), Italy	330, 336, 344, 348, 352	438, 452		351, 353, 362, 368, 372	
C3	*C. maliflora*	Villa Anelli (Vb), Italy	346, 352	442, 458		345, 372	
C4	*C. pitardii*	Kyushu University, Japan	344, 356	452	217, 227	364	
C5	*C. crapnelliana*	Villa Anelli (Vb), Italy	342, 346	476*	213	349	
C6	*C. saluenensis*	Villa Anelli (Vb), Italy	342, 346	458, 472*	217, 225	356, 376	
C7	*C. sinesis* var. *sinesis*	Villa Anelli (Vb), Italy	344, 346	449, 452	219, 227, 231	366	
C8	*C. tsaii*	Villa Anelli (Vb), Italy	346, 348, 366	460	211	349, 356, 358, 368	
C9	*C. granthamiana*	Villa Anelli (Vb), Italy	336, 340, 344	444, 450, 452, 454	223	353, 360, 378	
C10	*C. assimilis*	Villa Anelli (Vb), Italy	344, 346	444	211, 215		
C11	*C. cuspidata*	Villa Anelli (Vb), Italy	346, 356	460	203*	354	
C12	*C. japonica* var. *simplex*	Villa Anelli (Vb), Italy	346, 350	448	225, 244*	364	
C13	*C. sinesis* var. *assamica*	Villa Anelli (Vb), Italy	356	454	229, 237*	356, 366	
C14	*C. hongkongensis*	Villa Anelli (Vb), Italy	352			364, 372	
C15	*C. reticulata*	Villa Anelli (Vb), Italy	340, 346, 350, 352	444, 448, 452, 458, 462	211, 215, 229	356, 366	
C16	*C. sasanqua*	Villa Anelli (Vb), Italy	330, 336, 346, 348, 350	438, 444, 452	215, 221	353, 360, 364, 372	
C17	*C. kissii*	Villa Anelli (Vb), Italy	346	444	221	360	
C18	*C. japonica*	Kyushu University, Japan	348, 358	446, 450	219, 250*	360, 364	
C19	*C. sasanqua*	Kyushu University, Japan	344, 352, 360	446, 452	221	351, 368, 372, 380	
C21	*C. japonica* var. *rusticana*	Kyushu University, Japan	354	446	225	358, 360	
C22	*C. japonica* var. *intermedia*	Kyushu University, Japan	346, 358	448, 456	227	368	
C23	*C. japonica* var. *hozanensis*	Kyushu University, Japan	346	446	229	364	
C24	*C. chrysantha n. 325*	Kyushu University, Japan	346			354	
C25	*C. chrysantha n. 323*	Kyushu University, Japan	348			360	

C. japonica cultivars

C26	‘Tricolor’	Villa Anelli (Vb), Italy	354, 366	450	227, 233	364, 374	0.47
C27	‘Paolina Maggi’	Villa Pallavicino, (Vb), Italy	366	450	227, 233	366, 374	0.36
C28	‘Imperator’	Villa Anelli (Vb), Italy	348, 368	450	227, 233	360, 364	0.42
C29	‘Kallista’	Villa Anelli (Vb), Italy	348, 354	450	227	354, 364	0.48
C30	‘Angela Cocchi’	Villa Anelli (Vb), Italy	344, 348, 354	450	227	356, 362	0.47
C31	‘Humilis’	Villa Anelli (Vb), Italy	366	448	233	366, 374	0.26
C32	‘Virginia Franco’	Villa Anelli (Vb), Italy	354, 365*	450	227, 233	364, 374	0.45
C33	‘Gigantea’	Villa Anelli (Vb), Italy	354, 358	450	227, 231	356	0.50
C34	‘Preston Rose’	Villa Pascià, (Vb), Italy	352, 358	450	231, 249	358, 364	0.37
C35	‘Mathotiana Alba’	Villa Anelli (Vb), Italy	354	450	227	360, 366	0.37
C36	‘Mathotiana Rosea’	Villa Anelli (Vb), Italy	342	448	225	362	0.10
C37	‘Fimbriata’	Villa Anelli (Vb), Italy	354, 358	450	227, 231	356, 364	0.56
C38	‘Camilla Hebert’	Villa Anelli (Vb), Italy	348, 366	450	227, 233	356, 374	0.43
C39	‘Marchesa Margherita Sevesi’	Villa Anelli (Vb), Italy	348, 350	450	227	358, 366	0.37
C40	‘Roma Risorta’	Villa Anelli (Vb), Italy	348, 354	450	227	354, 364	0.48
C41	‘Alba Piena’	Villa Anelli (Vb), Italy	354	450	231, 241	358, 370	0.34
C42	‘Magnoliaeflora’	Villa Anelli (Vb), Italy	354, 366	450	227, 233	356, 366	0.46
C43	‘Vergine di Collebeato’	Villa Anelli (Vb), Italy	348, 354	450, 462	227	354, 364	0.51
C44	‘Lavinia Maggi Alba’	Villa Anelli (Vb), Italy	348, 352	450	227, 249	354, 370	0.38
C45	‘Lavinia Maggi’	Villa Anelli (Vb), Italy	350, 358	450	227, 249	366	0.37
C46	‘Lavinia Maggi Rosea’	Villa Anelli (Vb), Italy	358	450, 460	231, 241	364	0.38
C47	‘Oki-no-nami’	Villa Anelli (Vb), Italy	350, 358	450	227	366	0.36
C48	‘Elegans’	Villa Anelli (Vb), Italy	354, 358	444, 452	227, 231	356, 364	0.42
C49	‘Variegata’	Villa Wuhrer (Vb), Italy	350, 358	450	227	366	0.36
C50	‘Donckelaeri’	Villa Anelli (Vb), Italy	348, 354	450, 462	227	354, 364	0.51
C51	‘Incarnata’	Villa Anelli (Vb), Italy	344, 366	450	227, 233	364, 374	0.42
C52	‘Vittorio	Villa Anelli	354, 366	450	227, 233	362, 366	0.44

	Emanuele II’	(Vb), Italy					
C53	‘Otome-tsubaki’	Villa Anelli (Vb), Italy				354, 362	0.07
C54	‘Albino Botti’	Villa Anelli (Vb), Italy	354, 358	450	227, 231	356, 364	0.56
C55	‘Anemonaeflora’	Villa Anelli (Vb), Italy	354, 366	450	227, 233	364, 374	0.47
C56	‘Rawesiana’	La Margotta Nursery (Vb), Italy	358, 362	450	227, 231	364, 370	0.45
C57	‘Nazionale’	Rusca Garden, Locarno, Switzerland	358	450, 462	231, 241	354, 356	0.43
C58	‘Duchesse de Nantes’	Floricoltura Ratti Nursery (Vb), Italy	352, 354	450	227, 231	350*, 352*	0.41
C59	‘Bella d’Etruria’	Rusca Garden, Locarno, Switzerland	344, 348, 354	450	227	354, 364	0.49
C60	‘Covina’	Floricoltura Ratti Nursery (Vb), Italy	346, 358	450, 462	227, 231	356, 364	0.53
C61	‘Caryophilliflora Major’	Botanic Garden Madre Isle (Vb), Italy	352, 358	450	231, 249	356, 358	0.36
C62	‘Amalia Servi’	Rusca Garden, Locarno, Switzerland	348, 354	450, 462	227	362, 364	0.50
C63	‘Madame de Streakaloff’	La Margotta Nursery (Vb), Italy	354, 358	448, 450	231, 241	362, 364	0.47
C64	‘Gran Sultano’	Villa Wuhrer (Vb), Italy	354, 358	450	227, 231	358, 364	0.52
C65	‘Parvula’	La Margotta Nursery (Vb), Italy	348, 354	450, 462	227	362, 364	0.50
C66	‘Il Garofolo’	Rusca Garden, Locarno, Switzerland	350, 360	450	227, 245*	358, 366	0.33
C67	‘Antonietta Colnaghi’	Villa Ferrari (Vb), Italy	348, 354	450, 462	227	356, 362	0.49
C68	‘6019’	Rusca Garden, Locarno, Switzerland	356, 358	450	231, 241	354, 356	0.41
C69	‘12B’	Botanic Park, Brissago Isle, Switzerland			231, 241	354, 356	0.18
C70	‘14A'	Botanic Park, Brissago Isle, Switzerland	356	444, 450	231, 233	354, 356	0.39
C71	‘Gloria del Verbano’	Botanic Garden Madre Isle (Vb), Italy			231, 241	354, 356	0.18
C72	‘General Bem’	Rusca Garden, Locarno, Switzerland	346, 354	444, 450	225, 227	356, 362	0.45
C73	‘Calypso Vera’	Botanic Garden	354	450	227	362	0.42

C74	'Gloria delle Isole Borromee'	Madre Isle (Vb), Italy La Margotta Nursery (Vb), Italy	344, 350	450	231, 241	356, 364	0.40
C75	'Cruciata'	Floricoltura Ratti Nursery (Vb), Italy	347, 357*	444, 462	227, 231	362, 364	0.32
C76	'Bella di Pisa'	Rusca Garden, Locarno, Switzerland	347, 353*	448, 450	227	354, 356	0.38
C77	'Castagnola'	Rusca Garden, Locarno, Switzerland	348, 354	444, 450	227	356, 362	0.48
C78	'Daniel Webster'	Rusca Garden, Locarno, Switzerland	356, 358	450, 462	231, 241	354, 356	0.44
C79	'Amelia Brozzoni'	Rusca Garden, Locarno, Switzerland	344, 350		227	364	0.20
C80	'Oscar Borrini'	Rusca Garden, Locarno, Switzerland	344, 348, 354	450	227	354, 364	0.49
C81	'8B'	Botanic Park, Brissago Isle, Switzerland	354, 356	444, 450	233, 241	366	0.36
C82	'16B'	Botanic Park, Brissago Isle, Switzerland	356, 358	444, 450	231, 241	354, 364	0.44
C83	'6015'	Rusca Garden, Locarno, Switzerland	356, 366	450	233, 241	360, 364	0.34
C84	'11B'	Botanic Park, Brissago Isle, Switzerland	348, 358	450	227, 231	354, 364	0.52
C85	'Carlotta Papudoff'	Villa Taranto (Vb), Italy	356, 358	450, 462	231, 241	354, 356	0.44
C86	'6017'	Rusca Garden, Locarno, Switzerland			227, 231	356, 362	0.24
C87	'Frederic Alba'	Villa Taranto (Vb), Italy	348	450, 460	219, 227	360, 376	0.33
C88	'13B'	Botanic Park, Brissago Isle, Switzerland	348, 354	450, 462	225, 227	356, 364	0.53
C. sasanqua cultivars							
C89	'Hi no hakama'	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 342, 348, 350, 354, 362, 364	438, 444, 452, 456	215, 224	353, 361, 382	0.53
C90	'Hana jiman'	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 336, 342, 348, 356, 364	438, 452		353, 361, 365, 369, 378	0.37
C91	'Jean May'	Savioli F.lli	331, 336,	438, 444,		351, 353,	0.41

		Azienda Florovivaistica (Vb), Italy	338, 344, 350, 354, 364	452, 456		367	
C92	‘Cleopatra White’	Floricoltura Lago Maggiore (Vb), Italy	331, 348, 350, 354, 368	438, 448, 452, 456	215, 222	351, 353, 382	0.50
C93	‘Versicolor Sawada’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 336, 344, 348, 350, 356, 368	438, 444, 452, 470	213, 220	351, 353, 361, 365, 382	0.61
C94	‘FLM8’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 336, 342, 364	438, 444, 452, 470	215, 220	353, 361, 382	0.54
C96	‘Jennifer Susan’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 348, 350, 352, 354, 358	438, 448, 452, 470	220, 222, 226	351, 353, 361, 372	0.61
C97	‘Kogyoky’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 336, 352, 356, 362	438, 452, 470	215, 220	353, 361, 365, 382	0.53
C98	‘Plantation pink’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 342, 348, 350, 356, 360, 364	438, 446, 452, 456, 470	215, 224	351, 353, 365, 378	0.55
C99	‘Vicomte de Noailles’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 344, 348, 350, 362, 364, 368	438, 444, 452, 470	215, 224	351, 353, 361, 382	0.58
C101	‘Asakura’	Floricoltura Lago Maggiore (Vb), Italy	331, 338, 342, 348, 350, 356	438, 452, 456, 470	220	353, 365, 369, 372	0.51
C104	‘Presidente Antonio Sevesi’	Floricoltura Lago Maggiore (Vb), Italy	331, 336, 342, 348, 364	438, 452, 470	213, 220, 238*	353, 361, 365, 384, 397	0.49
C105	‘White Doves Benten’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 336, 338, 344, 350, 354	438, 452, 456, 470			0.33
C106	‘Sasanqua Rubra’	Vivaio Eisenhut, San Nazzaro, Switzerland	338, 344, 348, 350, 360, 374	438, 444, 452, 470			0.29
C109	‘Setsugekka’	Floricoltura Lago Maggiore (Vb), Italy	331, 342, 348, 350, 354, 358, 368	438, 444, 452, 470		353, 369, 382	0.47
C110	‘Totenko’	Vivaio Eisenhut, San Nazzaro, Switzerland	336, 342, 350, 358	438, 444, 448, 452			0.24
C112	‘FLM10’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 336, 352, 364, 368	438, 444, 452, 470			0.30
C113	‘Navajo’	Compagnia del Lago (Vb), Italy	331, 336, 342, 348,	438, 452, 470	215, 222	353, 369, 378	0.52

C116	‘Little pearl’	Vivaio Eisenhut, San Nazzaro, Switzerland	350, 352 331, 340, 350, 352, 354, 358	438, 444, 452, 470			0.33
C117	‘Cleopatra Sawada’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 348, 350, 354, 368	438, 448, 452, 456, 470	215, 220	351, 353, 382	0.61
C119	‘Marta Piffaretti’	Floricoltura Lago Maggiore (Vb), Italy	331, 340, 350, 352, 354, 358	438, 444, 452, 470	215, 222, 226	351, 353, 372, 374, 382	0.61
C120	‘Hinode gumo’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 344, 348, 350, 354, 358	438, 444, 452, 470	220, 222		0.48
C121	‘Daydream’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 344, 346, 348, 356, 364	438, 452, 456, 470			0.31
C122	‘Agnes o solomon’	Savioli F.lli Azienda Florovivaistica (Vb), Italy	336, 338, 350, 354, 364	438, 444, 452, 470	222	353, 369	0.42
C123	‘New down’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 348, 350, 354, 368	438, 448, 452, 456, 470	215, 222	351, 353	0.56
C125	‘Narumi-gata’	Floricoltura Lago Maggiore (Vb), Italy	331, 336, 340, 350, 352, 356, 360, 362	438, 452, 470	215, 224		0.39
C126	‘Hina yuki’	Floricoltura Lago Maggiore (Vb), Italy	356, 358	442, 448	230, 240	355, 357	0.06
C127	‘Maiden’s blush’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 344, 350, 354, 364	438, 444, 452, 470	215, 222		0.44
C128	‘Momozono nishiki’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 336, 342, 344, 350	438, 444, 452, 470	220, 222	351, 353, 382	0.56
C129	‘Fanny’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 338, 344, 348, 368	438, 448, 452, 456, 470	215, 220		0.45
C130	‘Autumn dawn’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 336, 348, 356, 358, 362	438, 452, 470	215, 224	353, 361, 365, 372, 382	0.53
C131	‘Isoli’	Compagnia del Lago (Vb), Italy	331, 336, 342, 348, 356, 364	438, 444, 452, 470	213, 220, 222	353, 361, 378, 384, 397	0.57
C132	‘Beatrice Emily’	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 344, 350, 354	438, 452, 470	220, 222	353, 365, 372	0.51
C. x *vernalis* cultivars							
C20	‘Gaisen’	Kyushu University, Japan	336, 346, 348	448, 452	213, 219, 221	353, 360, 362, 380	0.31

C95	'Yuletide'	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 344, 350, 352, 356	438, 446, 452, 470		351, 353, 359, 361, 369, 382	0.46
C100	'Hiryu nishiki'	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 348, 350, 352	438, 446, 452, 470		353, 359, 361	0.39
C108	'Ginryu'	Compagnia del Lago (Vb), Italy	331, 350, 368	438, 470	220	353, 372, 374	0.36
C111	'Star above star'	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 348, 350, 368	438, 448, 470	220, 226	353, 363*, 372	0.42
C114	'Hiryu'	Savioli F.lli Azienda Florovivaistica (Vb), Italy	356, 358	442, 448	230, 240	353, 355, 357	0.12
C115	'Vernalis egao'	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 348, 350, 368	438, 470	217, 220	353, 361, 372, 374	0.42
C. x *hiemalis* cultivars							
C107	'Bonanza'	Compagnia del Lago (Vb), Italy	331, 346, 348, 354	444, 452, 456	215, 220, 228		0.35
C118	'Sparkling Burugundy'	Compagnia del Lago (Vb), Italy	331, 346, 348, 354, 358	438, 444, 452, 456, 470	215, 222, 226	351, 353, 372, 374, 380, 382	0.62
C124	'Kanjiro'	Savioli F.lli Azienda Florovivaistica (Vb), Italy	331, 348, 350, 352, 354, 358, 374	438, 448, 452, 456, 470	215, 222, 228	351, 353, 372, 376	0.60
C. hybrida cultivars							
C102	'Winter's dream'	Vivaio Eisenhut, San Nazzaro, Switzerland	331, 348, 354, 360	438, 444, 452, 456, 470		351, 353, 367, 378, 382, 384	0.47
C103	'Winter's star'	Vivaio Eisenhut, San Nazzaro, Switzerland	344, 348, 350, 354, 358	438, 452, 470		353, 367, 380, 382	0.39

*Sequence Tagged Microsatellite Site (STMS) specific band.

Table 2. Allelic frequency of each microsatellite within *C. japonica* , *C. sasanqua* , *C.* x *vernalis* , *C.* x *hiemalis* , *C. hybrida* cultivars and all the sample set.

		MSCJAF37					
		C. japonica cultivars	*C. sasanqua* cultivars	*C.* x *vernalis* cultivars	*C.* x *hiemalis* cultivars	*C. hybrida* cultivars	All accessions
STMS allele	Size	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
1	330						0.01
2	331		0.16*	0.20*	0.19*	0.11	0.09
3	336		0.08	0.04			0.05
4	338		0.03				0.01
5	340		0.02				0.01
6	342	0.01	0.06				0.03
7	344	0.05	0.06	0.05		0.11	0.06
8	346	0.02	0.01	0.04	0.13		0.05
9	347	0.02					0.01
10	348	0.16	0.10	0.16	0.19*	0.22*	0.13*
11	350	0.06	0.12	0.20*	0.06	0.11	0.10
12	352	0.04	0.04	0.08	0.06		0.05
13	353	0.01					0.01
14	354	0.26*	0.08		0.19*	0.22*	0.12
15	356	0.06	0.05	0.08			0.05
16	357	0.01					0.01
17	358	0.19	0.04	0.04	0.13	0.11	0.09
18	360	0.01	0.02			0.11	0.01
19	362	0.01	0.03				0.01
20	364		0.06				0.03
21	365	0.01					0.01
22	366	0.08					0.02
23	368	0.01	0.04	0.12			0.03
24	374		0.01		0.06		0.01
		MSCJAH46					
		C. japonica cultivars	*C. sasanqua* cultivars	*C.* x *vernalis* cultivars	*C.* x *hiemalis* cultivars	*C. hybrida* cultivars	All accessions
STMS allele	Size	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
1	438		0.25*	0.26*	0.15	0.25*	0.15
2	442		0.01	0.05			0.01
3	444	0.09	0.12		0.15	0.13	0.11
4	446		0.02	0.11			0.03
5	448	0.05	0.06	0.16	0.08		0.07
6	450	0.68*					0.20*
7	452	0.03	0.24	0.16	0.23*	0.25*	0.17
8	454						0.01
9	456		0.09		0.23*	0.13	0.05
10	458						0.01
11	460	0.03					0.01
12	462	0.14					0.04
13	470		0.22	0.26*	0.15	0.25*	0.13
14	472						0.01
15	476						0.01

		MSCJAF25					
		C. japonica cultivars	*C. sasanqua* cultivars	*C.* x *vernalis* cultivars	*C.* x *hiemalis* cultivars	*C. hybrida* cultivars	All accessions
STMS allele	Size	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
1	200						0.01
2	203						0.01
3	211						0.01
4	213		0.06	0.10			0.02
5	215		0.29*		0.33*		0.10
6	217			0.10			0.02
7	219	0.01		0.10			0.02
8	220		0.29*	0.30*	0.11		0.09
9	221			0.10			0.02
10	222		0.23		0.22		0.07
11	223						0.01
12	224		0.10				0.02
13	225	0.03					0.03
14	226		0.04	0.14	0.11		0.02
15	227	0.42*					0.23*
16	228				0.22		0.01
17	229						0.01
18	230			0.14			0.01
19	231	0.24					0.08
20	233	0.13					0.06
21	237						0.01
22	238		0.02				0.01
23	240			0.14			0.01
24	241	0.13					0.06
25	244						0.01
26	245	0.01					0.01
27	249	0.04					0.02
28	250						0.01
		MSCJAH38					
		C. japonica cultivars	*C. sasanqua* cultivars	*C.* x *vernalis* cultivars	*C.* x *hiemalis* cultivars	*C. hybrida* cultivars	All accessions
STMS allele	Size	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
1	349						0.01
2	350	0.01					0.01
3	351		0.12	0.04	0.20*	0.10	0.05
4	352	0.01					0.01
5	353		0.27*	0.27*	0.20*	0.20*	0.12*
6	354	0.15					0.08
7	355		0.01	0.04			0.01
8	356	0.20					0.09
9	357		0.01	0.04			0.01

10	358	0.05					0.03
11	359			0.08			0.01
12	360	0.03		0.04			0.04
13	361		0.12	0.12			0.04
14	362	0.11		0.04			0.05
15	363			0.04			0.01
16	364	0.25*					0.12*
17	365		0.10				0.03
18	366	0.09					0.05
19	367		0.02			0.20*	0.01
20	368						0.02
21	369		0.06	0.04			0.02
22	370	0.03					0.01
23	372		0.06	0.12	0.20*		0.05
24	374	0.06	0.02	0.08	0.10		0.04
25	376	0.01			0.10		0.01
26	378		0.05			0.10	0.02
27	380			0.04	0.10	0.10	0.01
28	382		0.13	0.04	0.10	0.20*	0.05
29	384		0.02			0.10	0.01
30	397		0.02				0.01

* Sequence Tagged Microsatellite Site (STMS) alleles with the highest frequency (Freq.).

Table 3. Number of unique banding patterns in the studied cultivars and species of *Camellia* obtained by means of four STMS primer pairs, singularly and joined. In brackets, the number of banding patterns per number of accessions typed is reported.

	Total	*C. japonica* cultivars	*C. sasanqua* cultivars	*C.* x *vernalis* cultivars	*C.* x *hiemalis* cultivars	*C. hybrida* cultivars
N. of Accessions	*132 genotypes*	*63 genotypes*	*33 genotypes*	*7 genotypes*	*3 genotypes*	*2 genotypes*
MSCJAF37	55 (0.41)	26 (0.41)	16 (0.48)	4 (0.57)	1 (0.33)	2 (1.00)
MSCJAH46	22 (0.17)	7 (0.11)	4 (0.12)	4 (0.57)	3 (1.00)	2 (1.00)
MSCJAF25	30 (0.23)	10 (0.16)	9 (0.27)	5 (0.71)	2 (0.67)	1 (0.50)
MSCJAH38	22 (0.17)	13 (0.21)	4 (0.12)	3 (0.43)	2 (0.67)	2 (1.00)
Total	49 (0.37)	25 (0.39)	13 (0.39)	3 (0.43)	1 (0.33)	2 (1.00)

Table 4. Analysis of molecular variance. Twenty four species and 107 camellias cultivars were investigated. The analysis was based on STMSs markers on all the samples (a.) and on *C. japonica*, *C. sasanqua*, *C.* x *vernalis*, *C.* x *hiemalis* and *C. hybrida* cultivars (b.). Levels of significance were based on 1000 interation steps (SS, sums of squares; MS, means squares; %, proportion of genetic variability, fixation index; *P*, level of significance).

a.

Level of variation	d.f.	SS	MS	%	Φ_{ST}	*P*
Among populations	5	263.27	52.65	33.53	0.335	0.001
Within populations	126	665.90	5.28	66.47		0.001

b.

Level of variation	d.f.	SS	MS	%	Φ_{ST}	*P*
Among populations	4	227.82	56.96	40.09	0.401	0.001
Within populations	103	553.35	5.16	59.91		0.001